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Growth Regulators and Chemicals Stimulate Germination of Leafy Spurge (*Euphorbia esula*) Seeds

Michael E. Foley and Wun S. Chao*

Baseline information on inducing germination of dormant leafy spurge seeds with growth regulators and chemicals is lacking. This study was conducted to survey the effect of various substances on germination of leafy spurge seeds. The nontreated control seeds in this population were nearly fully imbibed in 3 h and displayed approximately 35% germination in 21 d under the normal alternating temperature of 20/30 C (16/8 h). Gibberellic acid (GA_3, 10 mM) induced 65% germination at constant temperatures of 20 and 30 C. The alternating temperature increased the effectiveness of 10 mM GA_3 with 94% germination, a twofold increase over the control. Nontreated seeds did not germinate at the constant temperatures, suggesting that alternating temperature acts via a GA-independent pathway. Kinetin at 0.1 to 1 mM was no more effective than the control, but a saturated solution of kinetin induced 73% germination. Ethephon at 0.01 to 1 mM induced 58 to 66% germination, although there was little response to different concentrations. Ethylene gas at 1 ppm stimulated germination to 77%, a 1.8-fold increase over the control. Germination of seeds incubated continuously in 1 and 10 mM nitrate displayed 35 and 40% germination, respectively. Seeds pulsed for 24 h with 100 mM nitrate displayed 58% germination after 21 d. Potassium phosphate–citrate buffer (pH 3.4) and its individual components induced 60 to 70% germination. Fluridone (10 and 100 μM), 1-naphthaleneacetic acid (NAA, 0.1 to 10 mM), and ethanol (0.2 to 15%) had no effect on germination, but subsequent elongation in the presence of NAA was inhibited because of swelling of the radicle. This research reveals that GA_3 is the most effective growth regulator for germination of dormant leafy spurge seeds, and its effect is independent of temperature.

Nomenclature: Leafy spurge, *Euphorbia esula* L. EPHES.

Key words: Dormancy, dormancy-breaking, germination, gibberellic acid, hormones, weed.

Leafy spurge is a noxious perennial weed of economic and ecological importance in natural and agro-ecosystems of the Northern Plains and Mountain West regions of the United States and Canada (DiTomaso 2000). Most of the recent research on this invasive plant has concentrated on control efforts through integrated pest management (Lym 2005). This plant has a remarkable ability to invade, spread, and persist because of vegetative reproduction from adventitious crown and root buds and sexual reproduction through seeds. Recent research on reproduction focused on regulatory mechanisms for bud dormancy (Chao et al. 2007).

Some research was conducted early in the 20th century on imbibition, germination, and longevity of leafy spurge seeds (Bakke 1936; Brown and Porter 1942; Hanson and Rudd 1933). The seed coat was not a barrier to water and oxygen uptake during imbibition, the germination capacity of seeds within a population was optimized by varying the temperature from 20 to 35 C on a 24- to 48-h basis, and seeds were viable in the soil for at least 3 yr. Early investigators recognized germination of freshly harvested seeds was limited, but they did not distinguish between viability and dormancy. Selleck et al. (1962) clarified that dormancy was likely responsible for the low germination capacity of freshly harvested seeds and observed that periods of seed drying and dark conditions during imbibition increased germination. One author (Foley 2008) further investigated the conditions of afterripening and determined that 12 to 24 wk at 30 C and 2.6% seed moisture promoted subsequent germination of leafy spurge seeds.

Many chemical and growth regulator treatments can break seed dormancy without afterripening (Bewley and Black 1994). The response of dormant seeds to various chemicals and growth regulators is species and even biotype specific. For example, germination of lettuce (*Lactuca sativa* L.) seeds was induced by treatment with cytokinin, whereas gibberellic acid (GA_3), which was effective on many other species, does not affect germination of this species (Kucera et al. 2005). The degree of dormancy in the population also determined responsiveness to chemical and growth regulator treatments (Hilhorst 1995). Highly dormant wild oat (*Avena fatua* L.) seeds responded rapidly to GA_3, but they must be partially afterripened to respond to nitrogenous compounds (Adkins and Simpson 1988). Wicks and Derscheid (1964) apparently recognized that 20 mM nitrate might stimulate leafy spurge seed germination; however, a stimulatory effect was not apparent because of lack of a water control.

Model plant systems, such as arabidopsis [*Arabidopsis thaliana* (L.) Heynh.], have contributed greatly to progress in understanding fundamental aspects of plant biology. Leafy spurge has been developed as a model perennial weed for fundamental research on biology and ecology and in particular on bud dormancy (Chao et al. 2005, 2007). Model plant status requires genomic resources, such as an expressed sequence tag (EST) database and microarrays, which allows simplified cloning of genes and examinations of global patterns of gene expression (Anderson et al. 2007; Chao et al. 2005). Effective use of leafy spurge microarrays to investigate bud dormancy was predicated on a reasonable level of baseline knowledge about the trait. Over a long period of time, many researchers provided descriptive and fundamental information on bud dormancy in leafy spurge that facilitated recent genomic investigations. In contrast, little baseline information exists on dormancy and germination of leafy spurge seeds. As part of a comprehensive study to develop our knowledge base on the biology of leafy spurge seeds and in preparation for genomic investigations, we determined the effect of various chemicals and plant growth substances on dormancy and germination of leafy spurge.

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* U.S. Department of Agriculture–Agricultural Research Service, Plant Science Research, 1605 Albrecht Boulevard, Fargo, ND 58105-5674. Corresponding author's E-mail: michael.foley@ars.usda.gov

Material and Methods

Plant Material and Germination. Leafy spurge has indeterminate flowering, and mature seeds dehisce naturally and forcefully from the fruit coats in the afternoon on warm, dry days. The plants were harvested in early morning to early afternoon at the peak of seed production in late June to early July 2006 from a field population in Fargo, ND (46°52′37″N, 96°47′23″W) to obtain a maximum amount of mature seeds. Seeds from this population are relatively dormant (Foley 2008). Plants were air dried in open paper bags for 7 to 10 d to facilitate seed dehiscence. All seeds were cleaned by hand, separated into six fractions by weight with a seed blower,[1] and then stored at −20 C to maintain dormancy. Seed fractionation was done because the populations harvested in the manner described above contained a proportion of nonviable and less mature seeds as judged by seed color (Wicks and Derscheid 1964) and our baseline germination tests on each fraction (unpublished data). To reduce the potential for confounding by nonviable and immature seeds, fraction 4 seeds were used for these experiments. Viability of seeds in fractions 3 to 6 was 95%, as judged by germination in the presence of 10 mM GA_3, and fraction 4 was selected for experiments because it contained a large proportion of the seeds (25%). Fraction 4 seeds obtained with a 3.8-cm seed blower separation tube, set at an airflow of 4.0 to 4.5 cm, averaged 3.0 mg $seed^{-1}$.

Seeds for each treatment were surface disinfected for 10 min with a 50% (v/v) solution of commercial bleach (6.25% NaOCl) containing a drop of Triton X-100 surfactant and rinsed 10 times for 1 to 2 min with sterile distilled water. The surface-disinfected seeds were redried in the laminar flow hood for about 2 h to their original fresh weight (FW) before imbibition for germination.

At least 20 seeds per treatment were placed in petri dishes (60 × 15 mm) containing 0.9 ml sterile distilled water or treatment solution and lined with one Whatman No. 1 filter paper. Distilled water in increments of 0.3 ml was added as needed to replace solution that evaporated from dishes during the course of the experiments. Except as noted for specific experiments with GA_3, dishes were maintained at an alternating temperature of 20/30 C (16/8 h; 23.3 C midpoint temperature) in a nonlighted incubator in boxes lined with wet paper towels to maintain high relative humidity. Germination, as judged visually by cracking of the seed coat, was normally determined daily throughout the 1 to 21 d of incubation.

Time Course of Imbibition. Seeds were surface disinfected and dried overnight as described above. The FW of 10 seeds per time period was determined. The seeds were placed in petri dishes and imbibed in water as described above for 0.5, 1, 3, and 6 h and 1, 3, 6, 9, 12, 15, 18, and 21 d. After periods of imbibition, the nongerminated seeds were rapidly blotted dry and weighed for FW determination. There were three replications per time period.

Treatments. *Gibberellic Acid*. Germination in response to 0, 0.1, 1, and 10 mM GA_3 was evaluated. GA_3[2] (10 mM) was dissolved in hot water while vortex mixing. After cooling, the solution was filter sterilized and serial diluted to prepare lower concentrations. Germination was conducted using the standard alternating conditions of temperature described above and at 20 and 30 C.

Cytokinin. Germination in response to 0, 0.1, 1, and 5 mM kinetin[3] was evaluated. A 1 mM solution was prepared in 1 N HCl and adjusted with 1 N KOH to pH 3.7. The solution was autoclaved and diluted to prepare lower concentrations. An approximate 5 mM saturated suspension of kinetin was obtained by adding 5 ml of 1 mM solution to the dish, evaporating the solution, and resuspending in 1 ml water.

Ethylene. Germination in response to 0, 0.01, 0.1, and 1 mM ethephon[4] was evaluated. Ethephon is an ethylene-generating compound that is stable in solution below pH 4; it breaks down, releasing ethylene, at neutral to alkaline pH (Warner and Leopold 1969). A 1 mM stock solution was prepared in sterile 100 mM MOPS buffer (pH 7.3) and serial diluted for lower concentrations. Freshly prepared solutions were added to dishes containing seeds. The dishes were sealed with parafilm, further sealed in a small clear plastic bag, further sealed in a slightly larger clear plastic bag, and finally placed on different shelves in an incubator. Parafilm and plastic bags surrounding the dishes were removed permanently after 7 d.

An effect of ethephon prompted experiments to determine the efficacy of ethylene gas. Germination in response to 0 and 1.0 ppm (μmol mol^{-1}) ethylene gas was evaluated. Sterile, clear, wide-mouth septa glass jars[5] (125 ml in size) were used for diluting ethylene gas and germination of seeds. An ethylene gas tank[6] fitted with a regulator coupled with a syringe adaptor[7] was used to set up the ethylene dilutions. To facilitate dilution of ethylene to a final concentration of 1.0 ppm from the original ethylene gas tank (10^6ppm), a 1 : 1,000 dilution was first prepared by injecting 125 μl of ethylene into a septa jar. Surface-disinfected seeds were placed in septa jars that contained sterile water and were lined with one Whatman No. 1 filter paper; then 125 μl of diluted ethylene (1 : 1,000) was injected. Controls were prepared in septa jars as described, but without ethylene. Ethylene was reinjected daily for the first 7 d and every 3 to 4 d thereafter. During reinjection, the controls (0 ppm) were also opened, mimicking the ethylene treatment.

Nitrate. Germination in response to 0, 1, 10, and 100 mM nitrate was evaluated. In preliminary experiments, we determined that 100 mM nitrate inhibited germination. Therefore, incubation at the high concentration of 100 mM was for 24 h followed by water for the duration of the experiment. KNO_3 (100 mM) was dissolved in water, adjusted to pH 3, and filter sterilized. Lower concentrations were prepared by serial dilution. The control solution was pH 3.

Ethanol. Germination in response to 0, 0.3, 3, 15, and 30% ethanol was evaluated. Ninety-five percent ethanol was diluted with sterile water to prepare the solutions.

Buffers. Germination in response to 0 (pH 3) and 50 mM citrate (pH 3.4), potassium phosphate (pH 3.4), and potassium phosphate–citrate (pH 3.4) was evaluated. Salts were dissolved in water, and the solutions were pH adjusted and filter sterilized.

Fluridone. Germination in response to 10 and 100 μM fluridone[8] was evaluated. The fluridone was dissolved in dimethysulfoxide with a final concentration of ≤ 0.1% in treatment and control solutions.

Auxin. Germination in response to 0.1, 1, and 10 mM 1-naphthaleneacetic acid (NAA) was evaluated. The NAA[9] was dissolved in ethanol with a final concentration of ≤ 10% in treatment and control solutions.

Statistical Analysis. There were three replications per treatment with each replication in a separate germination incubator and experiments were repeated at least once with similar results. Experiments with abscisic acid (ABA) were not repeated and are mentioned as unpublished data to coincide with experiments on the ABA biosynthesis inhibitor fluridone. Observed germination percentages for one representative experiment were fit to a three parameter logistic growth model $G = \Phi_1/\{1 + \exp[-(d - \Phi_2)/\Phi_3]\}$ where Φ_1 is the asymptotic germination rate (%), Φ_2 is the time (d) at which the populations reaches half its asymptotic germination, Φ_3 is the time (d) elapsed between the time the population reaches one-half the asymptotic germination rate and the time it reaches three-fourths germination rate, and d is time in days. Parameter estimates for an experiment were determined with the use of PROC NLIN in SAS and then compared with PROC GLM. Ninety-five percent confidence intervals are included with the germination curves.

Results and Discussion

Initial experiments to determine the effect of GA_3 on germination of dormant leafy spurge seeds were inconclusive. Unexpectedly, all concentrations of GA_3 generally provided only a small gain in germination compared with the control (data not shown). We hypothesized that the ethanol we used initially to solublize the GA_3 or chlorine in the surface disinfection solution inhibited germination. Alternatively, seeds might not take up sufficient hormone to readily bring the population to its full germination potential because they become imbibed during the surface disinfection (Cohn and Butera 1982; Grappin et al. 2000). To examine these alternatives, we determined the effect of ethanol on germination, investigated the time course of water uptake, and dried surface-disinfected seeds to remove water and degas chlorine before treatment with GA_3. Imbibition in 0.3 to 15% ethanol had no effect on germination; 30% ethanol inhibited germination (data not shown). In the aforementioned experiments with ethanol, germination of the control seeds in water was less than anticipated, which suggested an effect of surface disinfection on germination. Water uptake was measured to determine whether bleach solution might be absorbed during the process of surface disinfection. Leafy spurge seeds took up water very rapidly during imbibition (Figure 1). Seeds reached a maximum FW in about 3 h. About one-third of the water uptake occurred in 30 min, which is approximately the time required for the surface disinfection process. Thus, we dried surface-disinfected seeds for at least 2 h to their original FW before imbibition in treatment solutions.

Thereafter, GA_3 stimulated germination of dormant leafy spurge seeds incubated under the standard condition of alternating 20/30 C temperature (Figure 2A). GA_3 at 0.1 and 1.0 mM increased germination 1.1- and 1.4-fold, respectively, over the control, but 10 mM GA_3 induced 94% germination in 21 d, a twofold increase over the control (Table 1). Seeds treated with GA_3 containing 0.3 to 3% ethanol had nearly the same rate of germination as those treated with GA_3 in water (data not shown). This outcome is consistent with low concentrations of ethanol having no effect on germination of control and treated seeds.

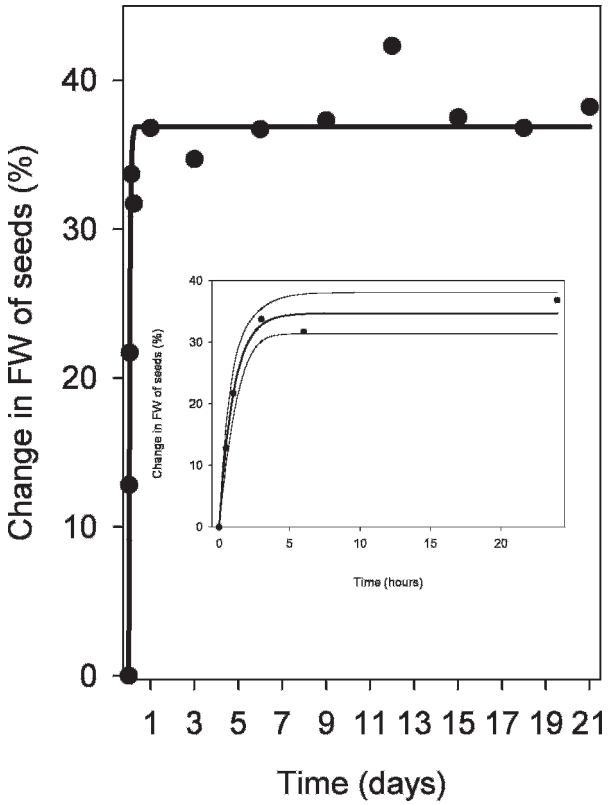

Figure 1. Time course of imbibition in water by nongerminated leafy spurge seeds. Initial seed fresh weight (FW) was 3.04 mg seed^{-1}. The insert in the figure shows changes in first 24 h. The 95% confidence intervals are depicted by the narrow lines. Percent change in FW of Seeds = 36.9[1 − exp(−21.2 × day)], R^2 = 0.96.

Germination of leafy spurge seeds was greater under alternating temperatures (e.g., 20/30 C) than under constant temperature (Brown and Porter 1942; Hanson and Rudd 1933). Because 10 mM GA_3 provided robust germination at an alternating temperature, we evaluated its effect on germination at constant temperature. As expected, nontreated control seeds did not germinate at 20 and 30 C (Figures 2B and 2C). Seeds treated with 0.1 mM GA_3 germinated 36 and 40%, respectively, at 20 and 30 C, and seeds treated with 10 mM GA_3 germinated 66 and 65%, respectively, at 20 and 30 C. The germination provided by 0.1 mM GA_3 at 20 and 30 C was about the same as control seeds germinated at the alternating temperature (Table 1). However, germination induced with 10 mM GA_3 at 20 and 30 C was about 30% less in absolute terms compared with germination at the same concentration using the alternating temperature (Figures 2A–C; Table 1). GA_3 and alternating temperature appear to have an additive effect; thus, they are not likely acting on the same response system. In any event, the reason GA_3 can compensate in part for lack of an alternating germination temperature is not apparent and will require further research.

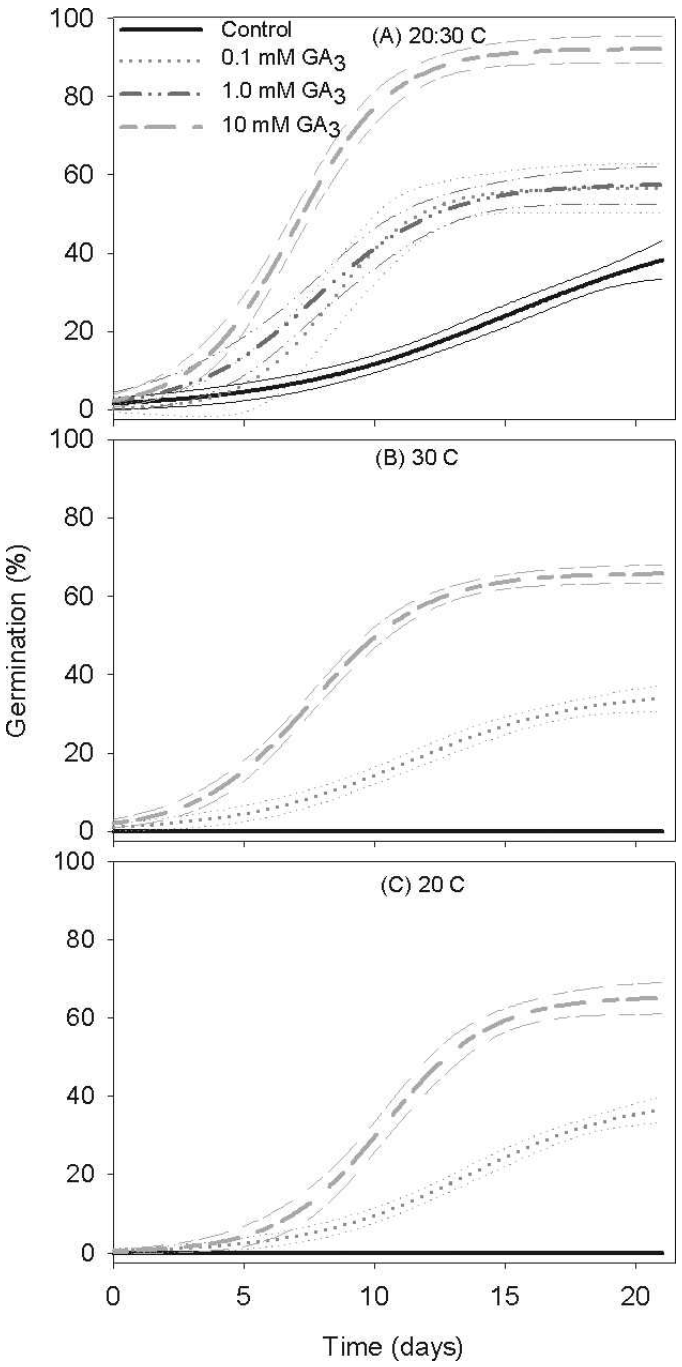

Figure 2. Predicted germination of leafy spurge seeds with gibberellic acid (GA_3) at temperatures of (A) 20/30 C (16/8 h), (B) 30 C, and (C) 20 C. The 95% confidence intervals are depicted by the narrow lines.

Kinetin at 0.1 and 1 mM had no effect on germination (Figure 3). Although some seeds respond to kinetin and other cytokinins, they are generally not considered active in overcoming seed dormancy (Thomas 1992). For leafy spurge, a saturated solution of approximately 5 mM induced 73% germination (Table 1), which would tend to support the idea that kinetin's lack of solubility at physiological pHs is a factor in its lack of efficacy (Cohn and Butera 1982).

In a manner that is largely independent of concentration, 0.01 to 1 mM ethephon stimulated 58 to 66% germination of leafy spurge seeds in 21 d (Figure 4A). Preliminary experiments with concentrations of 0.01 to 10 ppm ethylene gas revealed a stimulatory effect at 1 ppm ethylene gas (unpublished data). Follow-up experiments determined that 1 ppm stimulated 77% germination in 21 d (Figure 4B).

In a preliminary experiment with nitrate, we adjusted the solution pH with 50 mM potassium phosphate–citrate buffer

Table 1. Summary of Φ_1, Φ_2, Φ_3 and R^2 for germination treatments by category.[a] Values followed by the ± represent SE.

Treatment[b]	Φ_1[b]	Φ_2[c]	Φ_3[d]	R^2
	%	days		
GA_3, control	48.3 ± 9.9	15.1 ± 2.2	4.4 ± 0.93	0.81
10 mM	93.8 ± 1.4	7.1 ± 0.16	2.0 ± 0.14	0.94
1 mM	65.1 ± 2.0	9.6 ± 0.34	3.0 ± 0.27	0.90
0.1 mM	52.1 ± 2.8	9.1 ± 0.55	2.4 ± 0.46	0.69
Control (20 C)	—[c]	—	—	
10 mM (20 C)	65.8 ± 1.2	7.6 ± 0.20	2.2 ± 0.17	0.96
0.1 mM (20 C)	35.7 ± 2.7	11.3 ± 0.78	3.2 ± 0.54	0.86
Control (30 C)	—	—	—	
10 mM (30 C)	65.3 ± 2.2	10.4 ± 0.31	2.0 ± 0.25	0.92
0.1 mM (30 C)	39.8 ± 3.4	13.6 ± 0.77	3.1 ± 0.46	0.90
Kinetin, control	39.0 ± 6.0	13.9 ± 1.28	2.8 ± 0.80	0.70
0.1 mM	33.1 ± 2.8	12.6 ± 0.68	2.2 ± 0.53	0.79
1 mM	55.4 ± 19.2	18.1 ± 3.7	5.2 ± 1.1	0.83
5 mM	72.9 ± 2.7	12.3 ± 0.31	2.3 ± 0.23	0.95
Ethephon, control	29.4 ± 0.87	10.6 ± 0.25	1.5 ± 0.21	0.91
0.01 mM	57.6 ± 1.4	10.4 ± 0.22	2.0 ± 0.18	0.95
0.1 mM	66.0 ± 2.8	9.0 ± 0.44	2.4 ± 0.36	0.84
1 mM	65.7 ± 2.0	11.0 ± 0.28	2.2 ± 0.22	0.93
Ethylene, control	42.1 ± 1.9	10.0 ± 0.45	2.6 ± 0.36	0.87
1 ppm	77.4 ± 2.4	10.8 ± 0.31	3.0 ± 0.23	0.95
Nitrate, control	21.8 ± 1.1	11.6 ± 0.39	1.4 ± 0.33	0.84
1 mM	35.1 ± 3.3	11.2 ± 0.88	2.5 ± 0.68	0.72
10 mM	40.2 ± 1.6	9.5 ± 0.36	1.6 ± 0.30	0.87
100 mM (24 h)	57.5 ± 3.3	11.6 ± 0.56	3.0 ± 0.40	0.90
K^+ phosphate–citrate, control	38.9 ± 6.0	11.5 ± 1.7	3.6 ± 1.1	0.64
50 mM citrate	59.8 ± 2.3	7.9 ± 0.37	1.4 ± 0.32	0.83
50 mM K^+ phosphate	67.3 ± 1.7	9.2 ± 0.22	1.3 ± 0.19	0.93
50 mM K^+ phosphate citrate	69.6 ± 5.1	12.3 ± 0.68	2.9 ± 0.47	0.87

[a] Φ_1, asymptotic germination; Φ_2, time at which the populations reaches half its asymptotic germination; Φ_3, time elapsed between the time the population reaches one-half the asymptotic germination rate and the time it reaches three-fourths the germination rate; K^+ phosphate, potassium phosphate.
[b] Germinated at alternating temperature (20/30 C, 16/8 h) except as noted.
[c] No germination in 21 d.

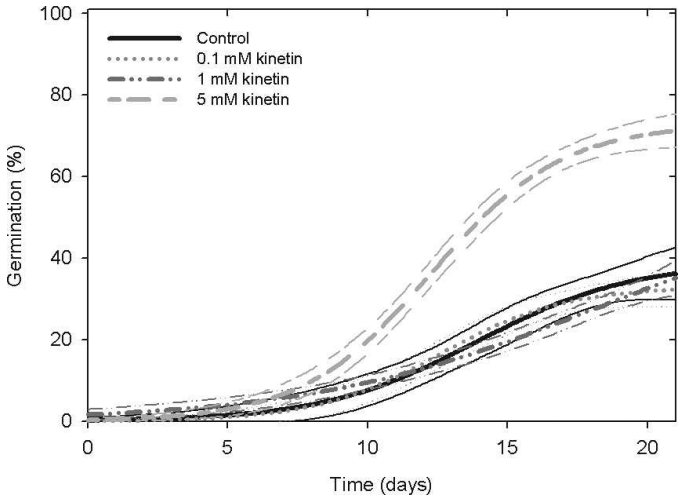

Figure 3. Predicted germination of leafy spurge seeds with kinetin. The 95% confidence intervals are depicted by the narrow lines.

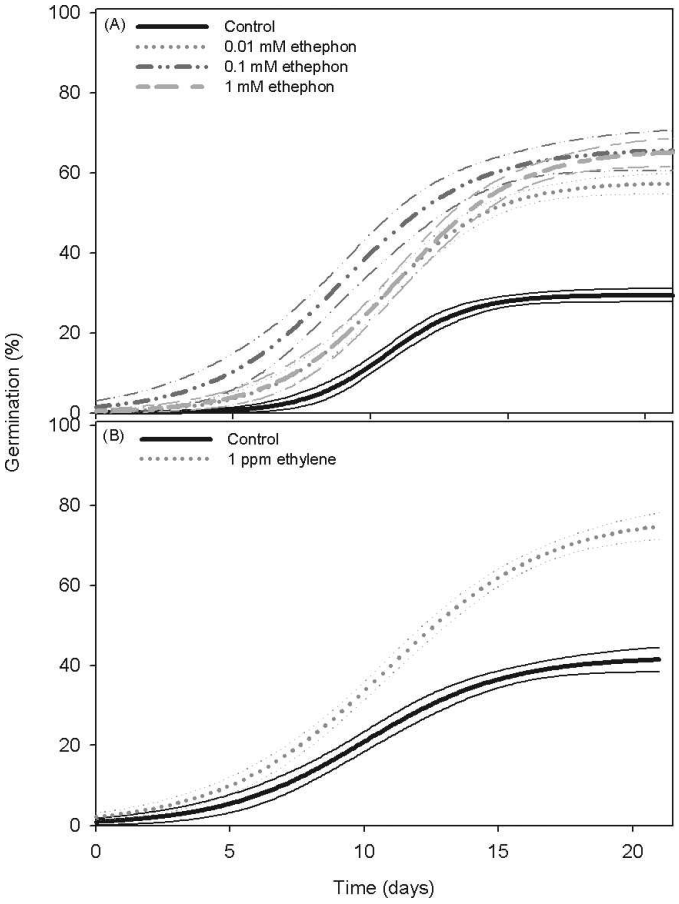

Figure 4. Predicted germination of leafy spurge seeds with (A) ethephon and (B) ethylene. The 95% confidence intervals are depicted by the narrow lines.

(pH 3.0). Under these conditions, the buffer control and 10 mM nitrate–treated seeds displayed 73 and 92% germination, respectively (data not shown). Germination of control seeds in buffer was much higher than we achieved for control seeds in water. Therefore, we determined the individual effect of nitrate dissolved in water and potassium phosphate–citrate buffer on germination. Nitrate at 1 and 10 mM induced 35 and 40% germination, respectively, at 21 d (Figure 5). These increases were relatively small when compared with 22% germination for the control (Table 1). A 24-h pulse with 100 mM nitrate increased germination modestly to 58% after 21 d (Figure 5). Fifty millimolar citrate, potassium phosphate, and potassium phosphate–citrate stimulated 60, 67, and 70% germination, respectively, of leafy spurge seeds after 21 d (Figure 6). Potassium phosphate and potassium phosphate–citrate stimulate germination to the same degree, whereas the citrate alone has slightly less effect on germination (Table 1). In any event, 100 mM nitrate, 50 mM citrate, and 50 mM potassium phosphate independently stimulate a moderate level of germination.

ABA and auxin are generally considered germination inhibitors. The auxin NAA had no effect on germination at concentrations of 0.1, 1, and 10 mM (data not shown), but subsequent elongation was inhibited by swelling of the radicle. ABA had no effect on germination of leafy spurge at concentrations of 0.1 and 1 mM, but inhibited germination at 10 mM (unpublished data). The ABA biosynthesis inhibitor fluridone at concentrations of 10 and 100 μM has no effect on germination (data not shown).

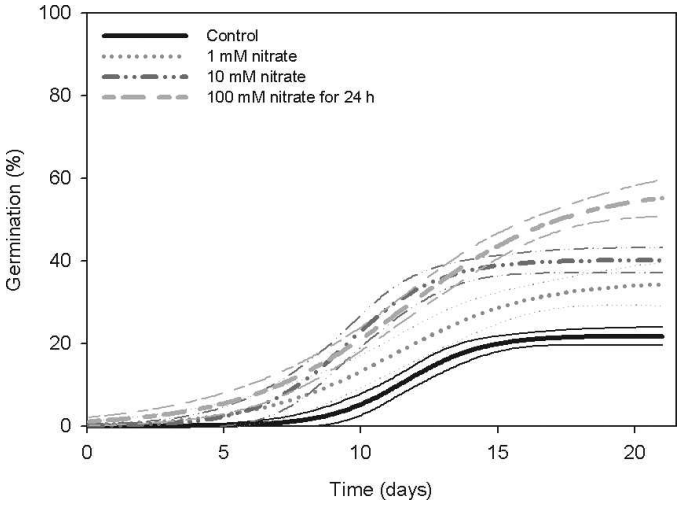

Figure 5. Predicted germination of leafy spurge seeds with nitrate. The 95% confidence intervals are depicted by the narrow lines.

Research on the effect of various chemical and plant growth substances on dormancy and germination of *Euphorbia* spp. has not been conducted (Baskin and Baskin 1998). In contrast, extensive descriptive and fundamental research has been conducted on arabidopsis (Ali-Rachedi et al. 2004; Finch-Savage et al. 2007; Kucera et al. 2005). Model plant status is making arabidopsis the standard for comparison in many biological investigations of eudicots. Because arabidopsis and leafy spurge are members of the phylogenetic clade Rosids and seed dormancy in both is classified as nondeep physiological dormancy (Baskin and Baskin 1998, Finch-Savage and Leubner-Metzger 2006), it has utility for gaining perspective on seed dormancy in leafy spurge and other species.

The current hormone balance hypothesis stipulates that the ABA : GA ratio, rather than absolute hormone levels, regulates physiological dormancy and germination (Cadman et al. 2006). ABA is a positive regulator of induction of dormancy and maintenance of dormancy in the imbibed state. GA_3 plays a role in promotion of germination by increasing the growth potential of the embryo, overcoming the mechanical constraints by weakening covering tissues surrounding the radicle, or both (Kucera et al. 2005). In addition, sensitivity to these hormones,

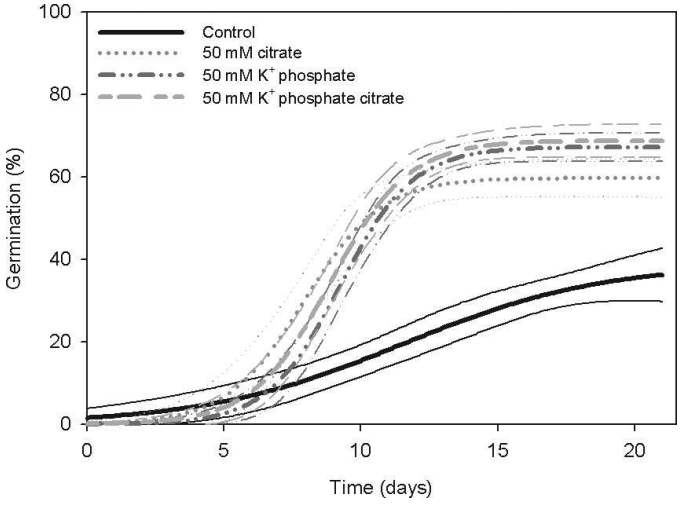

Figure 6. Predicted germination of leafy spurge seeds with potassium phosphate–citrate. The 95% confidence intervals are depicted by the narrow lines.

which changes during development and afterripening, plays a role in breaking dormancy and germination (Finch-Savage and Leubner-Metzger 2006). For robust induction of leafy spurge seed germination, a relatively high concentration of GA_3 was required (Figure 2A). This concentration apparently was sufficient to alter the internal ABA : GA balance in relation to the unknown sensitivity of dormant leafy spurge seeds to these hormones, thus stimulating germination (Ogawa et al. 2003).

ABA is thought to maintain seed dormancy, and treatments that reduce ABA levels facilitate germination (Grappin et al. 2000). Fluridone has been used to inhibit ABA biosynthesis in seeds of several species with varying effects on germination (da Silva et al. 2004; Gonai et al. 2004; Grappin et al. 2000; Le Page–Degivry and Garello 1992). The inhibitory effect on dormant arabidopsis seeds germinated at 20 to 27 C was reversed slowly by 10 μM fluridone and rapidly by the combination of fluridone and GA_3 (Ali-Rachedi et al. 2004). Similar effects were observed on thermo-inhibited lettuce seeds (Gonai et al. 2004). Fluridone did not stimulate germination of leafy spurge seeds. Follow-up studies will be needed to determine whether ABA has a role in leafy spurge seed dormancy (Hilhorst 1995).

The observation that 0.1 to 10 mM GA_3 could partially replace the requirement for alternating temperature to induce germination of leafy spurge demonstrates that the environment interacts with the hormone or hormone receptor to induce germination. Temperature fluctuations increase total germination of many species (Steinbauer and Grigsby 1957), although arabidopsis is not among those species (Ali-Rachedi et al. 2004). Leafy spurge would be a good model for investigating mechanisms underlying the role of alternating temperature and temperature–GA_3 interactions on germination with transcriptomic and proteomic approaches (Anderson et al. 2007; Chao et al. 2005). However, some research would be required on temperature amplitude, midpoint temperature, and light conditions for optimum germination (Murdoch et al. 1989) in that previous research gave no insight into these parameters for leafy spurge (Bowes and Thomas 1978; Brown and Porter 1942; Hanson and Rudd 1933; Selleck et al. 1962).

A recurrent theme of seed dormancy on the basis of research with mutant and wild-type arabidopsis and other plants, exogenous application of hormones and other chemicals, and hormone profiles is the role of hormone cross-talk on dormancy and germination (Finch-Savage and Leubner-Metzger 2006; Kucera et al. 2005). Hormonal and chemical signals form a complex network of information resulting in actions and interactions at many levels. Kinetin, ethylene, and nitrate act in a weakly to moderately positive way on germination of leafy spurge seeds. Their action is probably general in nature and the result of complex interactions (Finch-Savage et al. 2007; Kucera et al. 2005). For example, ethylene and nitrate appear to be negative regulators of ABA action in arabidopsis seeds by affecting metabolism or sensitivity (Alboresi et al. 2005; Chiwocha et al 2005; Ghassemian et al. 2000).

Citrate, potassium phosphate, and the combination act in a positive way on germination of leafy spurge seeds. We originally discovered this effect during the course of an experiment to determine the effect of pH on the efficacy of GA_3 (unpublished data). The 50 mM potassium phosphate–citrate buffer (pH 3.4) generally decreased the efficacy of GA_3 compared with GA_3 in water. Tian et al. (2003) observed a similar effect with dormant eastern gamagrass [*Tripsacum dactyloides* (L.) L.] seeds. However, leafy spurge seed germination was increased by the buffer compared with the water control. Subsequently, we sought to determine the efficacy of citrate because weak acids are known to break dormancy and stimulate germination of some seeds (Bewley and Black 1994; Cohn et al. 1989). Interestingly, potassium phosphate also stimulated germination. The role of inorganic phosphorous (P_i) and citrate has been investigated in wild oat. Levels of P_i in dry caryopses and the relative depth of dormancy are inversely correlated, suggesting P_i is involved in dormancy and germination (Jain et al. 1982). Citric acid and nitrate only induce germination in wild oat caryopses that had been afterripened for 9 wk, yet exceptionally dormant non-afterripened wild oat caryopses are highly responsive to GA_3 (Adkins et al. 1985). It is likely that the germination promotion by various chemically diverse substances is of a general nature involving alteration of membrane properties or lowering of pH to stimulate physiological activity (Cohn et al. 1989; Hilhorst 1998).

GA_3 is the most effective of the chemicals and growth regulators tested for inducing germination of dormant leafy spurge seeds. In addition, its activity is enhanced by an alternating germination temperature (Table 1). These observations support the hypothesis that GA is generally required for promotion of germination and environmental factors (e.g., temperature), can have an effect on induction of germination. These observations and the genomic resources now in place for leafy spurge (Anderson et al. 2007) set the stage to investigate the molecular basis for promotion of germination by alternating temperature and GA_3.

Sources of Materials

[1] South Dakota seed blower, Seedburo Equipment Co., 1022 W. Jackson Boulevard, Chicago, IL 60607.

[2] Gibberellic acid (G7645), Sigma, 3050 Spruce Street, St. Louis, MO 63103.

[3] Kinetin (K0735), Sigma, 3050 Spruce Street, St. Louis, MO 63103.

[4] Floral, Monterey Lawn and Garden Products, Inc., P.O. Box 35000, Fresno, CA, 93745.

[5] Wide-mouth septa glass jars (EW-99535-21), Cole-Parmer, Vernon Hills, IL 60061.

[6] Ethylene gas tank (04114 17), Scott Specialty Gases, Plumsteadville, PA 18949.

[7] Syringe adaptor (609010), Supelco, Bellefonte, PA 16823.

[8] Fluridone, Chem Service, Inc., P.O. Box 559, West Chester, PA 19381.

[9] 1-Naphthaleneacetic acid (N0640), Sigma, 3050 Spruce Street, St. Louis, MO 63103.

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